Meeting Report

BACTERIAL ENDOTOXINS: UNITY OR DIVERSITY OF HOST RESPONSE

Official satellite of the 4th International Congress of Immunology, Paris, 26 July, 1980

M. K. AGARWAL and J. PAILLARD

Centre National de la Recherche Scientifique, 15 rue de l'Ecole de Médecine, 75270 Paris Cédex 06, France

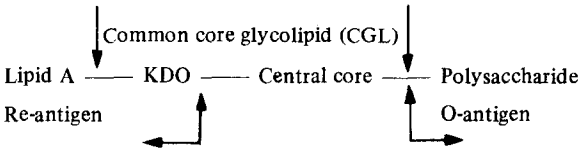
Received 21 October 1980

Two types of bacterial toxins have been classically recognised. The exotoxins are secreted by Gram-positive bacteria whose destruction by anti-biotherapy eventually leads to cessation of the toxin-induced pathogenesis in the host. Gram-negative bacteria are endowed with endotoxins consisting of the outer cell envelope that continues to provoke symptoms even after the bacterial cell ceases to exhibit growth or multiplication.

These endotoxins evoke a very wide variety of biological reactions, seem to be involved in shock, stress, septicaemia, infections, and are reported to affect almost all organ systems. Response to endotoxins, that can be either deleterious or beneficial to the well being of the host, has been studied with most tools available to the biologist but to date it has not been possible to establish a coherent picture of the manner in which so many different types of reactions are elicited by a large lipopolysaccharide (LPS) whose three-dimensional configuration is unknown. The efforts, hitherto, have been mostly limited to formulating models whereby effects on a particular process or organ may become comprehensible.

The workshop, summarised here, reviewed advances in endotoxin research over the past few years to see whether endotoxins attack one or a few sites initially, or whether the diversity of host response is an expression of simultaneous modulation of a number of targets in the organism.

Professor Nowotny (Philadelphia) discussed the basic problem of relating structure with function. At a purely chemical level, endotoxins are composed of a core (CGL) common to all Gram-negative bacterial cell walls, including 2-keto-3-deoxy-actonic acid (KDO) and lipid A, and polysaccharide 'O' antigens, linked covalently to lipid A, that confer species specificity, as shown below:



However, only 70% of the starting material may be subject to analysis by existing chemical procedures. Furthermore, polysaccharide deficient 'rough' mutants yield lipid A preparations that are neither chemically nor biologically identical. Heterogeneity within one class of preparation, from a single source, could also be detected by chromatography.

Endotoxins can either augment or suppress immune response and host resistance to infections, X-irradiation, tumour progression, in a time-dependent fashion. Biphasic response to endotoxins is also evident in proliferation of various cell types, activation of the reticuloendothelial system (RES), and mobilisation of overall immunocompetence, all of which underwrite the oscillating symptoms of the afore-mentioned disease syndromes following endotoxin administration. Since the cellular and humoral factors governing the overall prognosis are different, it was suggested that the interaction of heterogeneous components in endotoxins with a multifunctional immune system may initiate a diversity of host mechanisms governed by a unique time–response curve (Nowotny). It is however conceivable that only a few, initial, endogenous mediators, released after endotoxin–RES interaction, may secondarily produce an illusion of diversity depending upon the length of time required to measure change in the given process.

Dr Clumeck (Brussels) presented data to show that

high IgG and IgM titres against the O-antigen are associated with clinical remission of bacteraemia. IgG antibody to CGL, too, appears to have protective activity. The two may act synergistically where the former prepare bacteria for clearance from the blood and the latter for serum cidal action.

Dr Jirillo (Bari) argued that antibodies against lipid A are high in a population from areas endemic for typhoid fever and absent from a non-endemic population; antibody levels drop during antibiotic therapy. High anti-lipid A agglutinin activity is evident in urinary tract infection, but not in chronic pyelonephritis in children, due possibly to their local production in the kidney in the latter disease which can be provoked by lipid A in experimental animals. A serum factor that inhibits antibody–lipid A interaction may modulate the beneficial or detrimental effects of this antibody. However, isolated lipid A or CGL are poor immunogens, the surface of pathogenic bacilli contains little or no lipid A determinants (Clumeck) and unexposed lipid A remains embedded in the lipophilic membrane (Nowotny).

Professor di Luzio (New Orleans) raised the important point of correlation between various phases of a disease and systemic endotoxaemia, and the perpetual problem where contamination by even trace amounts of endotoxin may account for the therapeutic efficacy fallaciously attributed to a drug. Dr Wright (London) answered that the most sensitive limulus assay can detect endotoxin from some 100 lysed organisms whereas only 30/ml of Gram-negative bacteria are found in clinical septicaemia. Furthermore, 10^{-14} g endotoxin can be detected in the mouse but 10^{-7} g are required for manifestations of endotoxicosis; thus, contamination of a pharmacological agent is perhaps below the level where any sort of endotoxic effects could be observed.

LPS- or CGL-directed antibody can play a deleterious role by forming immune complexes leading to reactions akin to delayed-type hypersensitivity (Dr Kuwajima, Osaka). However, Dr Tlaskalova (Prague) showed that even in the absence of natural anti-LPS activity, in piglets deprived of maternal colostrum and raised in a germ-free environment, endotoxin reactivity progressed as evident by pyrogenicity, neutropenia, elevation in resistance to infections, altered blood coagulation.

Dr Hofman (Prague) provided evidence that LPS, a preferential activator of bone marrow derived B-lymphocytes, will stimulate lymphoid cells obtained from the liver and the marrow during the 114 day intrauterine life of pig foetuses; spleen cells responded to both B and T stimulators from day 50 of gestation, whereas thymus derived T-lymphocytes were transformed only by T-mitogens. T-cells can respond very impressively to LPS if they are pretreated with neuraminidase although it could not be ascertained whether this exposes LPS receptors on the cell surface or alters charge for the LPS. On the other hand, Dr Michalek (Birmingham) showed that although the germ-free mice are resistant to endotoxin lethality, B-cells derived from these animals are fully responsive to LPS in terms of adjuvanticity, immunogenicity, and mitogenesis. Rather, the resistance to LPS toxicity in conventional mice would seem to reside in the presence of suppressor T-cells in the spleen that depress B-cell response. Thus, either the absence of T-cells, or the presence of a large number of macrophages releasing a noxious mediator, would determine host susceptibility to LPS.

Endotoxins provoke cytotoxicity, decrease in the number of nucleated cells and increase in red blood cells in the bone marrow (Dr Yoshida, Morioka). Generation of a procoagulant by marrow cells leads to disseminated intravascular coagulation or DIVC (Dr Hirata, Morioka). Since different pharmacological agents did not influence these various reactions to the same extent, it was argued that endotoxins may hit multiple sites within the various cell types constituting the RES (Yoshida). The interaction of endotoxin with bone marrow-derived cells leads to the genesis of a large number of other mediators, such as histamine, serotonin, prostacycline, thromboxane, prostaglandins, which have diverse and far-reaching consequences on host homeostasis, and may play a determining role in the outcome of endotoxicosis (Dr Proctor).

As to the mechanism of cell damage, Dr Morrisson (Atlanta) demonstrated time- and temperature-dependent interaction of LPS with platelet membranes followed by possible insertion, at random or at designated receptor sites, of lipid A into the membrane bilayer, and eventual calcium translocation across the cell. However, very toxic 'smooth' LPS will bind less well than noxious 'rough' LPS, and insertion takes several hours whereas irreversible cell damage ensues in the first 60 min. It is furthermore difficult to conceive the applicability of this reasoning in vivo where lipid A remains unexposed on the cell wall of pathogenic bacteria.

At the metabolic level, blood glucose and total

body carbohydrate depletion are the most consistent reactions to endotoxin poisoning. Dr Spitzer (New Orleans) demonstrated adipocyte insulin receptors as possible sites for modulation of increased sensitivity to insulin during endotoxicosis. Dr Filkins (Maywood) suggested not only an insulinomimetic action of endotoxin but also release of monokines, macrophage insulin-like activity (MILA) and macrophage insulin-releasing activity (MIRA), in response to LPS all of which would lead to profound glucose dysregulation. Somatomedins, MILA, and a glucocorticoid antagonising factor (GAF) may, in fact, be identical. Dr Agarwal (Paris) pointed out that endotoxin–glucocorticoid interaction is actually a double-edged sword since either protection or sensitization to LPS can ensue, depending upon the time when the hormone is administered in relation to the toxin. Moreover, if mediators released from the RES sensitize to endotoxin lethality, it is paradoxical to find spleen involution at a time when the hormone sensitizes to LPS, although splenectomy by itself increases resistance to endotoxins (Agarwal).

The immunogencity, the large size of the LPS, elicitation of various mediators, induction of plasmacytoma (Hollander, New York), modification of response to LPS by glucan (Di Luzio), and many other reactions, all provide unequivocal support for the age-old concept that endotoxins react preferentially with the RES derived from the embryonic mesoderm. Dr Abernathy (Washington) was the only proponent of the possibility that LPS may alter membrane-bound Na,K-ATPase directly in isolated hepatocytes of endodermal origin. Inhibition of bile formation after endotoxin in vivo could not be correlated with normal

Scheme 1
Schematic representation of various host responses during endotoxicosis

	FIRST TARGET	MEDIATOR	SECOND TARGET	EFFECT
MESODERM → RES IN THE HOST	White blood cells	Leucocyte endogenous mediator	Various cells	Fever, Hypergammaglobulinemia, Leucocytosis
	Monocytes	Leucocytic pyrogen	Liver	Serum amyloid A synthesis, Secondary amyloidosis
	Peritoneal exudates	Endogenous pyrogen	Fever centres	Fever
	Bone marrow	Procoagulants	Complement	DIVC
	Liver RES	Monokines, GAF	Hepatocytes	Enzymatic dysfunction
B-cells	Spleen lymphocytes	Lymphokines	Macrophage Fc receptor	LPS nonresponder → Responder
	Granulocytes, Macrophages	Histamine & Serotonin.	Various cells	Vasoactive imbalance
		Procoagulants	Complement	Thrombosis
		MIRA, MILA	Liver, pancreas adipocyte etc.	Glucose, glycogen dysregulation
		Lysosomes	Various cells	Kinin release, degradation products
	Neutrophils	Prostaglandins	Various cells	Hypertension, ischemia, hypoxia
	Platelets	Thromboxane	Vaisseaux	Altered permeability
T-cells	Spleen	Lymphokines	Suppressor T-cells	Adjuvanticity, Immunogenicity, Mitogenicity
	Thymocytes	? ? ?	ibid	
ENDODERM →	HEPATOCYTES	cAMP	none	Bile production

Abbreviations are explained in the text

tests for parenchymal cell function (Abernathy) and absence of endotoxin in hepatocytes, although liver RES activity is altered under similar conditons (Agarwal).

In conclusion, the rather simplified scheme shown here attempts to summarise the diversity of host reactions after interaction of LPS with RES of mesodermal origin which releases a battery of mediators culminating in a series of chain reactions much like the coagulation cascade and cAMP both of which are influenced by endotoxins. Specialised reactions, reported in journals of immunology, pharmacology, biochemistry, infections and medicine, may proceed through surveillance of the RES responding to endotoxins. Surprisingly, the time required to measure change in RES function is actually longer than some of the subsequent effects after LPS–RES mediation (Agarwal). Future challenges include the narrowing down of the choice of possibilities by chemical identification of various mediators, physicochemical analysis of events on the surface and within the cell in contact with endotoxin, and the establishment of a time sequence which will eliminate secondary effects of endotoxins from primary site(s) of attack.

Acknowledgements

The opening address of the workshop was kindly given by Professor J. Dry, President, University of Paris VI Pierre et Marie Curie. Grateful appreciation is due to Professor J. Dausset, President of the 4th International Congress of Immunology, for administrative support and publicity. For financial assistance, thanks are due to the Paris offices of the following organisations: Laboratoire Clin-Midy; Laboratoire Glaxo; LKB Instruments SA; Merck, Sharp and Dohme, Chibret; Packard Instruments SA; Smith, Kline and French; and Congrès Services. Institut Pasteur, Paris, graciously provided the conference amphitheatre and associated facilities along with reception personnel.

Full proceedings of the meeting are to be published by Elsevier/North-Holland, Amsterdam, New York.